

Amendments to the Specification:

Please insert the paragraph below directly after the title on page 1:

STATEMENT REGARDING SEQUENCE LISTING SUBMITTED ON CD-ROM

The Sequence Listing associated with this application is provided on CD-ROM in lieu of a paper copy, and is hereby incorporated by reference into the specification. Three CD-ROMs are provided, containing identical copies of the sequence listing: CD-ROM No. 1 is labeled COPY 1, contains the file 471c14.app.txt which is 941 KB and created on November 10, 2003; CD-ROM No. 2 is labeled COPY 2, contains the file 471c14.app.txt which is 941 KB and created on November 10, 2003; CD-ROM No. 3 is labeled CRF (Computer Readable Form), contains the file 471c14.app.txt which is 941 KB and created on November 10, 2003.

Please replace the paragraph beginning at page 59, line 5 with the following redlined paragraph (or section):

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the LasergeneTM suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenies pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor* 11:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA* 80:726-730.

Please replace the paragraph beginning at page 62, line 21 with the following redlined paragraph (or section):

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from ~~GenBank~~GENBANK. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence.

Please replace the paragraph beginning at page 83, line 16 with the following redlined paragraph:

Other preferred adjuvants include MontanideTM ISA 720 (Seppic, France), SAF (Chiron, Emeryville, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Ribi ImmunoChem Research Inc., Hamilton, MT), RC-529 (Corixa, Seattle, WA) and Aminoalkyl glucosaminide 4-phosphates (AGPs).

Please replace the paragraph beginning at page 99, line 19, with the following redlined paragraph (or section):

Two-thousand clones from the above mentioned cDNA subtraction library were randomly picked and submitted to a round of PCR amplification. Briefly, 0.5 µl of glycerol stock solution was added to 99.5 µl of pcr MIX (80 µl H₂O, 10 µl 10X PCR Buffer, 6 µl 25 mM MgCl₂, 1 µl 10 mM dNTPs, 1 µl 100 mM M13 forward primer (CACGACGTTGTAAAACGACGG) (SEQ ID NO:688), 1 µl 100 mM M13 reverse primer (CACAGGAAACAGCTATGACC) (SEQ ID NO:689)), and 0.5 µl 5 u/ml Taq polymerase (primers provided by (Operon Technologies, Alameda, CA). The PCR amplification was run for

thirty cycles under the following conditions: 95°C for 5 min., 92°C for 30 sec., 57°C for 40 sec., 75°C for 2 min. and 75°C for 5 minutes.

Please replace the paragraph beginning at page 105, line 21 with the following redlined paragraph:

Human colon tumor antigens were obtained using SCID mouse passaged colon tumor RNA as follows. Human colon tumor was implanted in SCID mice and harvested, as described in Patent Application Serial No. 08/556,659 filed 11/13/95, U.S. Patent No. 5,986,170. First strand cDNA was synthesized from poly A+ RNA from three SCID mouse-passaged colon tumors using a Lambda ZAP ExpressTM cDNA synthesis kit (Stratagene, La Jolla, CA). The reactions were pooled and digested with RNase A, T1 and H to cleave the RNA and then treated with NaOH to degrade the RNA. The resulting cDNA was annealed with biotinylated (Vector Labs, Inc., Burlingame, CA) cDNA from a normal resting PBMC plasmid library (constructed from Superscript plasmid System, Gibco BRL), and subtracted with streptavidin by phenol/chloroform extraction. Second strand cDNA was synthesized from the subtracted first strand cDNA and digested with S1 nuclease (Gibco BRL). The cDNA was blunted with Pfu polymerase and EcoRI adaptors (Stratagene) were ligated to the ends. The cDNA was phosphorylated with T4 polynucleotide kinase, digested with restriction endonuclease XhoI, and size selected with Sephacryl S-400 (Sigma). Fractions were pooled, ligated to Lambda ZAP ExpressTM arms (Stratagene) and packaged with Gigapack Gold IIITM extract (Stratagene). Random plaques were picked, phagemid was excised, transformed into XL0LR cells (Stratagene) and resulting plasmid DNA (Qiagen Inc., Valencia, CA) was sequenced as described above.

Please replace the heading on page 109, line 7 with the following redlined heading:

~~SUBTRACTION~~SUBTRACTION

Please replace the paragraph beginning at page 109, line 11 with the following redlined paragraph:

Using the GibcoBRL Superscript Plasmid System with minor modifications, two cDNA libraries were created. The first library, referred to as CTCL, was prepared from a pool of mRNA samples from three colon adenocarcinoma tissue samples. Two of the samples were described as Duke's stage C and one as Duke's stage B. All three samples were grade III in histological status. A second library (referred to as DriverLibpcDNA3.1+) was prepared from a pool of normal tissues, namely liver, pancreas, skin, bone marrow, resting PBMC, stomach and brain. Both libraries were prepared using the manufacturer's instructions with the following modifications: an EcoRI-NotI 5' cDNA adapter was used instead of the provided reagent; the vector pCDNA3.1(+) (Invitrogen, Carlsbad, CA) was substituted for the pSPORT vector; and the ligated DNA molecules were transformed into ElectroMaxDH10B electrocompetent cells. Clones from the libraries were analyzed by restriction digest and sequencing to determine average insert size, quality of the library and complexity of the library. DNA was prepared from each library and digested.

Please replace the heading on page 113, line 1 with the following redlined heading:

Results from the PSORTII protein analysis program (University of California-Berkeley, Berkeley, CA) suggest that RECC is a cell surface protein containing 1 transmembrane domain. PSORTII predicts a cleavable signal peptide from amino acids 1-17, one transmembrane domain, a C-terminal cytoplasmic tail, amino acids 446-512, and a 44.4% likelihood of extracellular localization. The IDENTIFY analysis program (Stanford University, Palo Alto, CA) predicts four EGF-type signatures CACVPGY (SEQ ID NO:1130) and one rhesus blood group protein signature at stringency of one in 10^6 (no false positives expected).

Please replace the paragraph beginning at page 123, line 17 with the following redlined paragraph (or section):

Twenty milliliters of lysis buffer was added to the cell pellets and vortexed. To break open the *E. coli* cells, this mixture was then run through the French Press at a pressure of 16,000 psi. The cells were then centrifuged again and the supernatant and pellet were checked by SDS-PAGE for the partitioning of the recombinant protein. For proteins that localized to the cell pellet, the pellet was resuspended in 10 mM Tris pH 8.0, 1% CHAPS and the inclusion body pellet was washed and centrifuged again. This procedure was repeated twice more. The washed inclusion body pellet was solubilized with either 8 M urea or 6 M guanidine HCl containing 10 mM Tris pH 8.0 plus 10 mM imidazole. The solubilized protein was added to 5 ml of nickel-chelate resin (Qiagen) and incubated for 45 min to 1 hour at room temperature with continuous agitation. After incubation, the resin and protein mixture were poured through a disposable column and the flow through was collected. The column was then washed with 10-20 column volumes of the solubilization buffer. The antigen was then eluted from the column using 8M urea, 10 mM Tris pH 8.0 and 300 mM imidazole and collected in 3 ml fractions. A SDS-PAGE gel was run to determine which fractions to pool for further purification. As a final purification step, a strong anion exchange resin such as Hi-Prep Q (~~Biorad~~Bio-Rad, Hercules, CA), was equilibrated with the appropriate buffer and the pooled fractions from above were loaded onto the column. The antigen was eluted off of the column with an increasing salt gradient. Fractions were collected as the column was run and another SDS-PAGE gel was run to determine which fractions from the column to pool. The pooled fractions were dialyzed against 10 mM Tris pH 8.0. This material was then submitted to Quality Control for final release. The release criteria were purity as determined by SDS-PAGE or HPLC, concentration as determined by Lowry assay or Amino Acid Analysis, identity as determined by amino terminal protein sequence, and endotoxin level was determined by the Limulus (LAL) assay. The proteins were then vialled after filtration through a 0.22-micron filter and the antigens were frozen until needed for immunization.

Application No. 10/025,380
Reply to Office Action dated July 8, 2003

Please delete the section of the application entitled "Sequence Listing" immediately after the section of the specification entitled "Abstract of the Disclosure" on page 136 and in lieu of a new paper copy please insert the enclosed CD-ROM copy of the Sequence Listing therefor.